

**Conservation Genetics of Endangered Suckers of the Klamath  
region: Mitochondrial DNA**

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**Interim Report to  
Cooperative Agreement 9-FC-20-17650**

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## PRELIMINARY RESULTS

DNA was extracted from 326 individuals and analyzed for variation. Samples were initially screened with several sets of primers from the cytochrome b and ND4L genes, with those that amplify the ND4L gene selected because of ease of scoring and sequencing. Sixteen haplotypes were identified from 324 individuals (Table 1) with two samples not working (these will be tried again).

**Species identification.--** The distribution of haplotypes among morphotypes generated some interesting results. Most haplotypes (N=10) were rare, occurring in only one or two individuals, while the remaining six haplotypes were found in at least 12 individuals. Several morphotypes exhibited unique haplotypes that allowed them to be diagnosed relative to each other. Individuals identified as *C. rimiculus* always exhibited haplotypes I and L that were only rarely found in other morphotypes. Most *D. luxatus* (35 of 40) had haplotypes that were unique or rarely found in *Ch. brevirostris*. The remaining two morphotypes, *C. snyderi* and *Ch. brevirostris*, had high frequencies of the B haplotype, however, the upper Williamson River sample of *C. snyderi* exhibited a unique allele (F) in high frequency found nowhere else.

Phylogenetic analysis identified limited divergence among haplotypes as each differed from neighboring alleles by one or two mutation events (Figure 1). It is interesting to note that haplotypes within a morphotype tended to

cluster together, indicating that they were more similar to each other than to haplotypes found in other morphotypes.

(NOTE - The results above are based upon morphotypes provided by OSU. They have indicated some identification problems, mainly involving *Ch. brevirostris* and *C. snyderi*. Therefore discussion of relationships between morphotypes and mtDNA haplotypes is preliminary and should be interpreted cautiously.)

**Phylogenetic analysis.**-- To examine historical relationships of the four Klamath basin suckers relative to other catostomids, we obtained sequences from the entire cytochrome b and ND4L genes (ca. 1500 bp) from geographically adjacent forms and other species in the same tribe (Catostomini) and an outgroup (*Erimyzon*) from the Moxostomini. Phylogenetic analysis (Figure 2) provided generally good resolution as most nodes were supported upon resampling the data with replacement (e.g., exhibited high bootstrap values). This analysis indicated that most members of the genus *Catostomus* group together, with relationships within consistent with geographic and geological information. There are, however, notable exceptions, as *C. rimiculus* and *C. snyderi* possess sequences most similar to *Ch. brevirostris* and *D. luxatus*, and this group is closest to one containing *Ch. cujus* and *C. warnerensis*. Relationships among these two groups and

the *Catostomus* lineage are not strongly supported upon resampling, indicating additional characters are essential for determining if the Klamath basin group is more closely related to the *Chasmistes* or *Catostomus* lineages.

**Preliminary assessment.**-- Phylogenetic placement and relatively low levels of mtDNA divergence among these four morphotypes (when compared to other catostomids) is surprising given the existence of old fossils for some of these forms (Miller and Smith 1981). Therefore, it would appear all have been influenced by hybridization in the past, with recent isolation obtained by *C. rimiculus* and *D. luxatus*. Identity of haplotypes in *C. snyderi* and *Ch. brevirostris* likely indicates that gene exchange among these forms still occurs.

#### REFERENCE

Miller, R. R., and G. R. Smith. 1981. Distribution and evolution of *Chasmistes* (Pisces: Catostomidae) in western north America. Occ. Pap. Mus. Zool. Univ. Michigan 696:1-46.

Table 1. Distribution of mtDNA ND4L haplotypes by morphotype (provided by D. Markle, OSU). Question marks identify known difficulties with morphological identifications at the time of analysis. Individual genotypes appear on the web site maintained by Markle.

Allele	N	CHBR	DLUX	CRIM	CSNY
A	2	2	0	0	0
B	186	129	3	0	54
C	1	1	0	0	0
D	2	2	0	0	0
E	1	1	0	0	0
F	17	0	0	0	17
G	2	0	0	0	2
H	1	0	0	0	1
I	27	0	4?	23	1?
J	2	0	1	1	0
K	40	7	33	0	0
L	28	0	0	28	0
M	1	0	1	0	0
N	12	10	2	0	0
O	1	0	1	0	0
P	1	1	0	0	0
	324	153	41	52	74

Figure 1. Phylogeny of ND4L haplotypes. Letters are haplotypes identified in Table 1, with smaller fonts indicating rare haplotypes. Numbers on branches indicated the number of mutations between haplotypes.

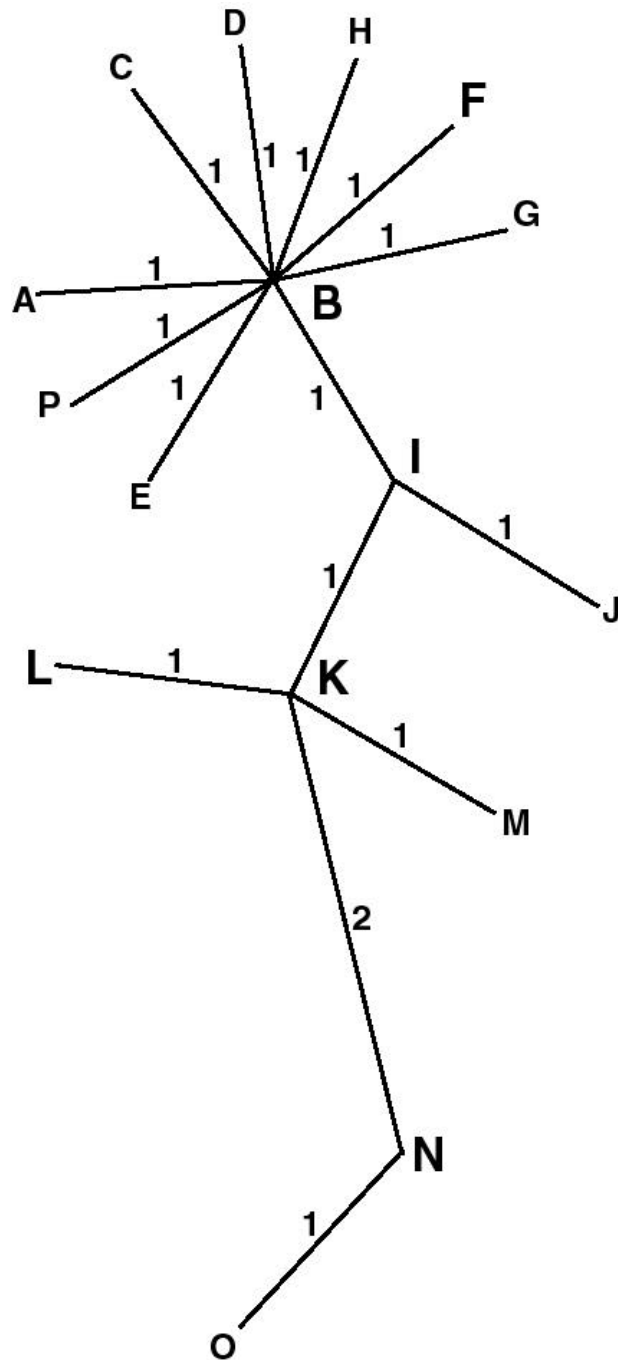


Figure 2. Phylogenetic relationships among catostomids as determined by sequences of cytb and ND4L obtained by neighbor-joining of Kimura two-parameter distances. Branch lengths are proportional, with scale provided. Numbers on branches identify the proportion of 1000 bootstrap replicates in which relationships in the grouping were found.

